Technical Report

Project Title: Investigating *Phytophthora sojae* Populations Across North Dakota Soybean Production Systems

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Research Overview and Objectives:

Background information and research gaps.

Phytophthora root and stem rot (PRSR) caused by the soilborne organism *Phytophthora sojae* is a significant threat to soybean production, leading to yield losses of over 40 million bushels annually across the United States and Canada. In North Dakota, soybean farmers rely on commercial varieties with resistance genes such as Rps 1c, 1k, 3a, and 6. However, recent studies have shown that certain pathotypes of *P. sojae* can overcome these resistance genes, reducing their effectiveness. Additionally, a related pathogen, *Phytophthora sansomeana*, has been identified in several Midwestern states but its presence in North Dakota remains undocumented. This proposal aims to address these issues by characterizing the diversity of P. sojae pathotypes in North Dakota, identifying the presence of *P. sansomeana*, and screening soybean breeding lines for resistance to prevalent pathotypes. This research will provide valuable insights into the virulence of *P. sojae* populations and inform breeding programs and farmer recommendations to enhance disease management strategies and improve soybean yields.

Research Objectives:

1. The diversity of *Phytophthora sojae* pathotypes present in North Dakota soybean fields will be determined
2. *Phytophthora sansomeana* may be identified in North Dakota soybean fields
3. The current status of the effectiveness of *Rps* genes against *P. sojae* will be determined
4. The soybean breeding efforts will be improved by targeting the most important pathotypes of *P. sojae*.
5. Soybean farmers will have access to improved recommendations to manage PRSR, which includes cultivar selection

Materials and Methods:

During the 2023 growing season, 147 soil samples were collected across North Dakota, with contributions from our research program, farmers, crop consultants, and NDSU Extension personnel. This collection process will continue through the 2024 and 2025 field seasons. Soil samples were subjected to a baiting technique to isolate Phytophthora species. The collected soil were ground, sieved, and placed into small cups or pots with holes in the bottom. These cups or pots were flooded for 24 to 48 hours and then drained and incubated at a constant temperature of 77°F for 14 days. Pots were then be flooded again for another 24 hours, and then leaf discs from a susceptible soybean variety, Sloan, were placed on top of the water and allowed to incubate. These leaf discs were then removed from the flooded soil and placed onto a selective growth media allowing for water mold pathogens to grow. Resulting cultures were isolated and placed onto new growth media. To confirm that isolates were Phytophthora, the cultures were plated onto Potato dextrose agar (PDA), which Phytophthora will not grow on. However, a very closely related group of pathogens called Pythiums will grow on PDA. These isolates were also examined under a microscope to evaluate their morphology. Suspected isolates of Phytophthora were then subjected to DNA extractions for further molecular confirmation. All isolates were then placed into long-term storage for future use.

To confirm the pathotype of each Phytophthora isolates, they will need to be inoculated onto 10 seedlings of soybean differential lines with known Rps genes. This work will allow for the characterization of the pathotype of each. However, prior to this work, adequate seed quantities are needed to run these assays. Each of these differentials has been growing under greenhouse conditions to increase the quantity to run these assays.

Research Findings/Outcomes:

From the research conducted during the 2023-24 season, a notable finding was the widespread presence of *Pythium* species across the collected soil samples. The majority of isolated species were identified as *Pythium* through either morphological or molecular methods. In contrast, only ten isolates have thus far been suspected of being *Phytophthora* through morphology. The limited number of *Phytophthora* isolates may be attributed to the dry conditions during the 2023 field season, which made it challenging to identify areas with high *Phytophthora* pressure. Alternatively, these results suggest that many suspected *Phytophthora* incidences might be more complex than previously thought, potentially involving a higher prevalence of *Pythium* species than expected. Further testing in subsequent years will be necessary to confirm whether this trend holds true for soybean production fields in North Dakota.

Disclosure of Inventions or Plant Varieties:

None

Discussion:

The preliminary findings from the 2023-24 season have revealed a widespread presence of *Pythium* species across the collected soil samples, with the majority of isolated species identified as *Pythium*. Only ten isolates have been confirmed to be *Phytophthora*. The limited number of *Phytophthora* isolates could be attributed to the dry conditions experienced during the 2023 field season, which may have hindered the identification of areas with high *Phytophthora* pressure. However, these results might also indicate a more complex scenario than previously anticipated, suggesting a potentially higher prevalence of *Pythium* species in suspected *Phytophthora* cases.

Despite the initial findings, it is crucial to note that this research is ongoing, with two more years of data collection planned to achieve the research objectives fully. The moist conditions so far in the 2024 season, combined with the widespread occurrence of seedling diseases, provide a positive outlook for a more comprehensive dataset in the coming year. The continuation of soil and plant sample collection, coupled with the pathotype characterization of *Phytophthora* isolates, will be essential in confirming the trends observed in 2023 and enhancing our understanding of *Phytophthora sojae* and Pythium populations in North Dakota.

These efforts are expected to yield better data, allowing for a more accurate assessment of the effectiveness of Rps genes and the identification of *Phytophthora sansomeana* if present. The outcomes of this research will be critical for informing soybean breeding programs and developing improved management recommendations for soybean farmers. By targeting the most important pathotypes of *P. sojae* and providing growers with up-to-date recommendations, this project aims to enhance disease management strategies and ultimately improve soybean yields in North Dakota.

Benefits to North Dakota Soybean Farmers and Industry:

The results of this study thus far allow farmers to better understand the pathogen presence within their fields. From this research, Pythium species appear to be more prevalent than previously believed. This impacts management strategies as there currently is no genetic resistance like Phytophthora Rps genes available commercially, and farmers may need to rely more on seed treatments to properly control seedling disease and stand-loss issues due to these different pathogens. However, further work is still needed to better understand the distribution and status of Phytophthora populations across the state in a different field season.
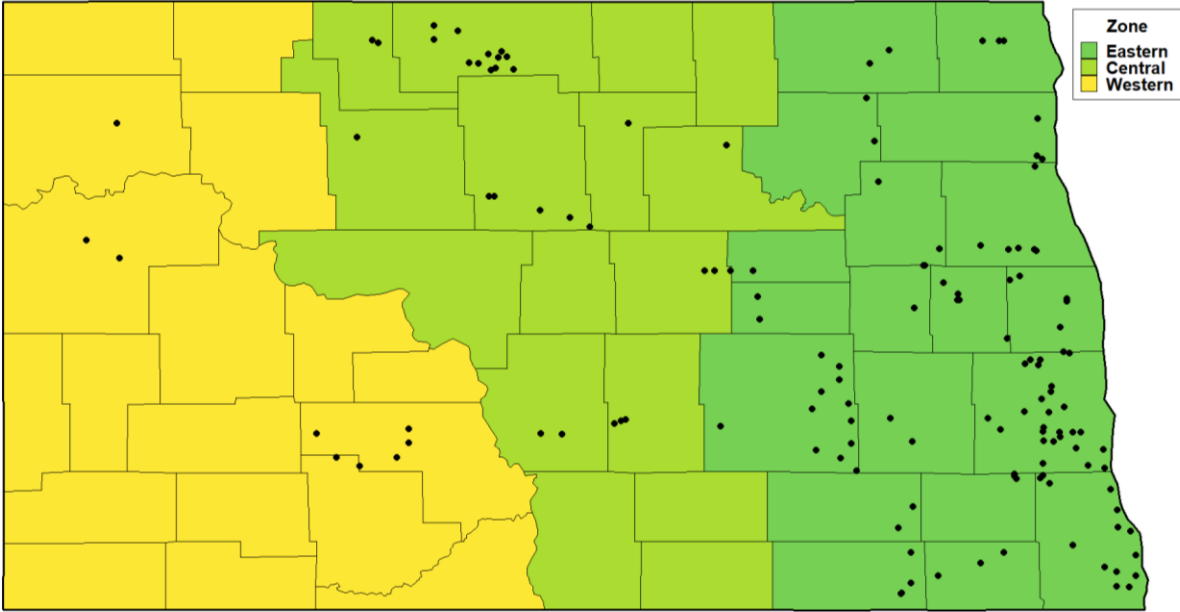
Acknowledgements:

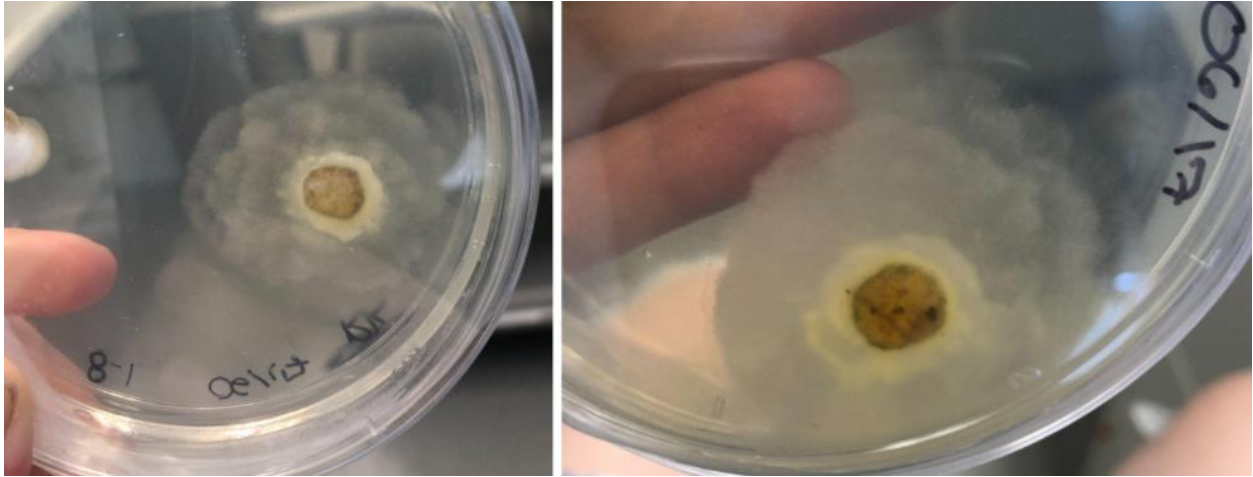**Figure 1.** Map of North Dakota with locations of soil samples collected for evaluation of *Phytophthora sojae* populations.

Figure 2. Oomycete growth from susceptible soybean leaf discs.